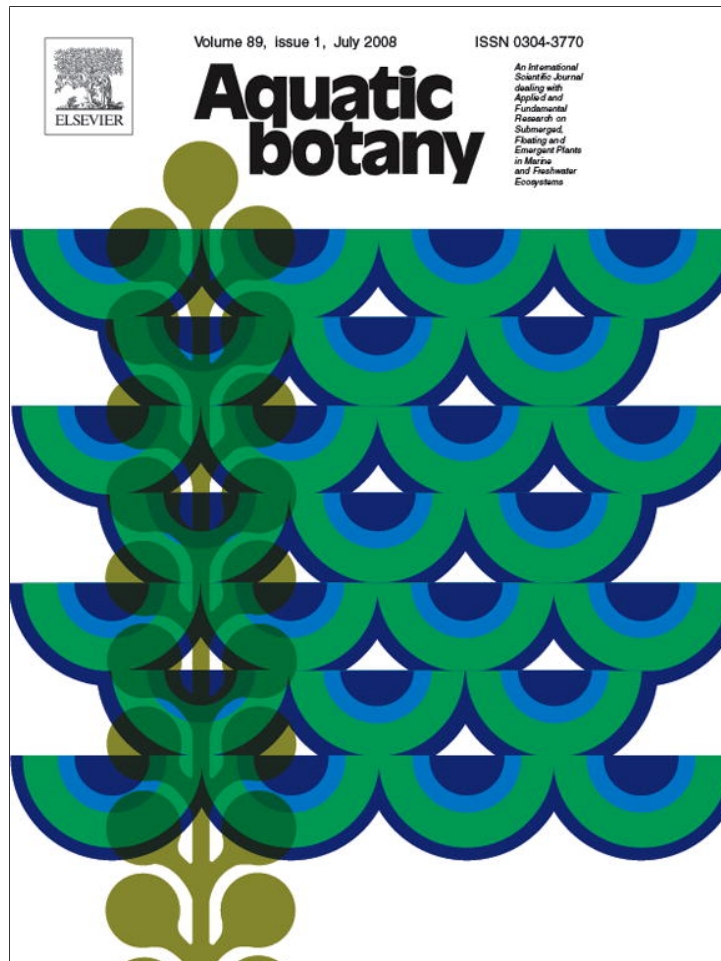


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Diurnal light curves and landscape-scale variation in photosynthetic characteristics of *Thalassia testudinum* in Florida Bay

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Received 6 June 2007; received in revised form 16 January 2008; accepted 17 January 2008

Available online 2 February 2008

Abstract

When using pulse-amplitude modulated (PAM) fluorometry to measure landscape-scale photosynthetic characteristics, diurnal variations in fluorescence during sampling may confound the assessment of the physiological condition. In this study, two photophysiological assessment techniques: Diurnal Yield and Diurnal Rapid Light Curve (RLC) were investigated in an attempt to incorporate the temporal and spatial scales of sampling into a physiological assessment of *Thalassia testudinum* in Florida Bay. Photosynthesis–irradiance (P–E) curves were calculated using both methods and the ability of each to predict the relationship between relative electron transport rates and irradiance was assessed. Both methods had limitations in providing consistent estimates of photosynthetic efficiency or capacity. The Diurnal Yield method produced unrealistically high predictions of photosynthetic capacity (relative electron transport rate (rETR_{max}), 417–1715) and saturation irradiance (I_k , 1045–4681 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). In contrast, the Diurnal RLC method generally produced predictions of rETR_{max} (100–200) and I_k (300–500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) which were similar to average values calculated from each day's RLCs. The Diurnal RLC method was unable to predict photosynthetic efficiency (α) only when ambient irradiances were continuously $>I_k$ during the sampling period. We believe that with sampling modifications in high-light or shallow environments, such as starting sampling earlier in the morning, extending sampling later in the day, or using the average α from each day's RLCs, that the Diurnal RLC method can produce representative estimates of rETR_{max}, α , and I_k , providing a method to characterize seagrass photosynthesis at the landscape-level. The Diurnal RLC method does not negate Diurnal variation but it produces a curve that incorporates the changing ambient light environment into the assessment of seagrass physiological status.

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Keywords: PAM fluorometry; Photosynthesis; Diurnal variation; Seagrasses; *Thalassia testudinum*

1. Introduction

Pulse-amplitude modulated (PAM) fluorometry provides almost instantaneous *in situ* measurements of a variety of photosynthetic characteristics, under ambient conditions. With the development of an underwater fluorometer, Diving PAM (Walz, Germany), it is now possible to study photosynthesis of aquatic organisms, such as seagrasses, without the use of gas-exchange enclosures (Beer et al., 1998). The measurement of chlorophyll fluorescence, emitted from photosystem II (PSII), provides insight into changes in photochemistry, and permits the study of effects of varying environmental conditions on

photosynthetic reactions (White and Critchley, 1999; Schreiber, 2004; Ralph and Gademann, 2005; Ralph et al., 2007). PAM fluorometry is also an attractive assessment tool because it is rapid, non-destructive, and can provide in-depth, quantitative physiological information about an organism.

In an initial study incorporating PAM fluorometry into a landscape-scale assessment of seagrass condition in Florida Bay, significant diurnal variation in chlorophyll fluorescence was detected (Durako and Kunzelman, 2002). The variability of the physiological signal was evident as significant negative slopes in regressions of effective and maximum (5 min dark adapted) quantum yields against ambient irradiance or time of day. Florida Bay is a subtropical lagoonal estuary, thus, resident organisms are exposed to relatively large ranges of irradiances and temperatures throughout the day. Photosynthesis can rapidly respond to changes in the light environment (MacIntyre

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et al., 2000) and the sensitivity of PAM fluorometry in detecting these rapid changes may result in highly variable physiological signals when assessing the physiological condition of seagrasses at the landscape scale. Belshe et al. (2007) further observed that rapid light curve (RLC) derived parameters (α and relative electron transport rate ($rETR_{max}$)) also exhibited diurnal variability, but the magnitude, direction and significance of the variations were inconsistent among basins and years. Statistical testing was unable to determine which time of the day was best suited for assessing seagrass photophysiological status. Belshe et al. (2007) concluded that when performing ecosystem-level assessments, sampling the entire spatial scale of interest provides more representative information than using only a time-restricted subsample, but that diurnal variation has to be accounted for in spatial comparisons.

Longstaff et al. (2002) investigated the accuracy of PAM fluorometry versus O_2 evolution techniques in assessing diel variability *in situ* photosynthetic rates. They took measurements with an automated O_2 exchange apparatus, performed RLCs and obtained point measurements of effective quantum yield over a diel (20 h) period. By constructing traditional photosynthesis–irradiance (P–E) curves with O_2 measurements and fluorescence-based diel light curves using what they termed the ‘Diel Yield’ and ‘Diel Rapid Light Curve’ methods, they were able to generate comparable curves that spanned the entire temporal scale of sampling. They found that under certain conditions and with some limitations (mainly at higher irradiance levels) PAM fluorescence could accurately assess photosynthetic rates of the simple laminate algae, *Ulva lactuca* L. Here, we tested the application of the Diel Yield and Diel RLC methods (termed here Diurnal Yield and Diurnal RLC because our measurements were obtained during daylight hours) in order to incorporate time-of-day considerations in conducting large-scale physiological assessments. The objective was to discern the usefulness of the two diel light curve methods described by Longstaff et al. (2002) for overcoming methodological and logistical constraints (i.e., <15 min to complete sampling at each station) inherent with landscape-scale ecological assessment and also to determine the two methods’ effectiveness in characterizing the physiological condition of the seagrass *Thalassia testudinum*.

2. Materials and methods

2.1. Study site

This study was conducted in Florida Bay (ca. 25°05′N, 81°45′W), a shallow lagoonal estuary at the southern tip of Florida, USA. The Bay is characterized by shallow basins (ca. <1 m) divided by carbonate mud banks and mangrove islands (Fourqurean and Robblee, 1999). As part of the Fish Habitat Assessment Program (see Durako et al., 2002 for more information on FHAP), 10 basins were sampled that lie within the borders of the Everglades National Park (ENP) (Table 1). The basins were chosen to represent the range of conditions within the bay. Each basin was divided into 27–33 tessellated hexagonal subunits, and one station was randomly chosen

within each subunit. This resulted in 275–330 stations that were randomly sampled throughout the Bay (see Hackney and Durako, 2004 for a map of sampling stations). Florida Bay is approximately 2000 km² and the sampled basins range in size from 5.8 to 62.4 km² (Durako et al., 2002). As a result of the large sample area, stations must be sampled systematically in order to minimize station-to-station travel time, yet it still takes an entire day (~0800 to 1700 h) to sample each basin. Because of navigational and safety concerns, FHAP sampling in the Bay can only be conducted during daylight.

2.2. Sampling technique

Photosynthetic characteristics were measured using an underwater fluorometer, Diving PAM (Walz, Germany), in 2002 (13–23 May) and 2004 (20–31 May), during the spring FHAP sampling. RLC were performed on four haphazardly chosen short shoots of *T. testudinum* at each station. The short shoots that were chosen were representative of the shoots observed at each station. A dark leaf clip (DIVING-LC) was attached to the middle of the rank 2 blade of each *T. testudinum* short shoot (Durako and Kunzelman, 2002). The leaf clip held the Diving PAM fiber optic 5 mm from the surface of the blade in 2002. This distance was reduced to 2 mm in 2004 in order to allow for a reduction in instrument gain to achieve a higher signal to noise ratio. Each RLC was initiated within 2–5 s after attaching the leaf clip to minimize dark acclimation (i.e. quasi-darkness yield, Ralph and Gademann, 2005). Leaves were exposed to eight incremental steps of irradiance ranging from 90 to 2060 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in 2002, and 5 to 1735 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in 2004. The reduction in irradiance levels in 2004 was due to slight damage to the fiber optic causing a decrease in light transmission at the same instrument settings. An effective quantum yield (Φ_{PSII}) measurement ($\Delta F/F_m'$) was taken at the beginning of each curve, before light was applied, and at the end of each 5 s irradiance step, resulting in nine yield measurements for each RLC performed. Each Φ_{PSII} measurement was used to calculate the relative electron transport rate through photosystem II using the equation recommended by Beer et al. (2001):

$$rETR = \Phi_{PSII} \times PAR \times AF \times 0.5$$

where PAR is the light generated by the internal halogen lamp of the Diving PAM, AF is the fraction of light absorbed by the leaf, and 0.5 assumes that the photons absorbed are equally partitioned between PSII and PSI (Genty et al., 1989). Due to time limitations at each station (28–33 stations were sampled each day, allowing <15 min station⁻¹), it was not possible to measure leaf absorption; therefore, AF was assigned a value of 1 (Beer et al., 2001), and relative electron transport rates are presented.

2.3. Diurnal light curves

To assess changes in photosynthesis in response to changing ambient irradiances, two types of light curves were calculated using a modification of what Longstaff et al. (2002) termed the

Table 1
Results from non-linear regressions of photosynthetic irradiance curves (P–E) generated with the Diel RLC and Diel Yield methods, including r^2 and level of significance for the regressions (P -values denoted as ≤ 0.05 , ≤ 0.01 and n/s = not significant) and the derived parameters α (dimensionless), $rETR_{max}$ (dimensionless), and I_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 10 basins sampled within Florida Bay in 2002 and 2004

Basin	Diurnal RLC method					Diurnal Yield method					Rapid light curves		
	r^2	P -value	α	$rETR_{max}$	I_k	r^2	P -value	α	$rETR_{max}$	I_k	α	$rETR_{max}$	I_k
2002													
Blackwater Sound BLK	0.97	**	0.41	125	309	0.99	**	0.38	887	2324	0.42 ± 0.05	98 ± 25	234
Calusa Key CAL	0.97	**	0.36	141	387	0.99	**	0.37	777	2122	0.39 ± 0.05	117 ± 29	301
Crane Key CRN	0.99	**	0.31	168	545	0.99	**	0.35	667	1893	0.32 ± 0.04	141 ± 18	442
Eagle Key EAG	0.97	**	0.32	152	469	0.99	**	0.37	579	1545	0.37 ± 0.07	118 ± 33	319
Johnson Key JON	0.98	**	0.21	133	646	0.99	**	0.37	1715	4681	0.38 ± 0.04	107 ± 18	282
Madeira Bay MAD	0.96	**	0.17	169	1018	0.99	**	0.35	1282	3692	0.32 ± 0.05	124 ± 26	389
Rabbit Key RAB	0.98	**	0.33	116	356	0.99	**	0.37	n/a	n/a	0.40 ± 0.03	94 ± 12	236
Rankin Lake RKN	0.96	**	0.21	200	939	0.99	**	0.34	n/a	n/a	0.32 ± 0.03	145 ± 27	455
Twin Key TWN	0.98	**	0.31	145	465	0.99	**	0.39	578	1477	0.39 ± 0.07	109 ± 25	280
Whipray Bay WHP	0.98	**	0.36	144	401	0.99	**	0.40	417	1045	0.39 ± 0.06	110 ± 24	282
2004													
Blackwater Sound BLK	0.92	**	0.32	173	547	0.99	**	0.37	655	1780	0.35 ± 0.05	132 ± 39	379
Calusa Key CAL	0.98	**	0.30	153	509	0.99	**	0.36	706	1969	0.30 ± 0.05	132 ± 23	440
Crane Key CRN	0.96	**	1651.31	118	0	0.98	**	0.29	787	2714	0.29 ± 0.06	106 ± 20	368
Eagle Key EAG	0.91	**	1.71	143	83	0.97	**	0.33	654	1964	0.24 ± 0.06	124 ± 31	521
Johnson Key JON	0.95	**	0.32	100	309	0.99	**	0.34	965	2826	0.36 ± 0.04	85 ± 18	239
Madeira Bay MAD	0.93	**	0.12	132	1124	0.98	**	0.37	763	2061	0.29 ± 0.05	116 ± 19	402
Rabbit Key RAB	0.94	**	0.23	133	583	0.99	**	0.35	1529	4392	0.37 ± 0.04	105 ± 25	286
Rankin Lake RKN	0.94	**	0.14	145	1058	0.99	**	0.39	n/a	n/a	0.36 ± 0.06	111 ± 24	309
Twin Key TWN	0.96	**	0.30	161	534	0.99	**	0.37	639	1716	0.33 ± 0.05	135 ± 27	411
Whipray Bay WHP	0.95	**	0.30	128	426	0.99	**	0.31	1108	3532	0.30 ± 0.05	114 ± 21	380

Also given are the mean and standard deviation for parameters (α and $rETR_{max}$) and calculated I_k for the rapid light curves from which both diel curve methods were derived. If the model failed to converge it is denoted as n/a.

Diel RLC and the Diel Yield methods. Longstaff et al. (2002) obtained measurements over a 20-h period, whereas, here RLCs were taken throughout the daily sample period (~ 0800 to 1700 h), along with measurements of the ambient irradiance at the seagrass canopy, measured using a quantum PAR scalar sensor (LiCor LI-193S). For the Diurnal RLC method, $rETR$ s were calculated for all nine effective quantum yield measurements in the RLC using the internally generated irradiances. The RLC produced using the internal actinic irradiance was used to interpolate $rETR$ at the measured ambient irradiance taken at the time of the RLC. Interpolated $rETR$'s and ambient irradiances were plotted to create a diurnal P–E curve for each sample basin and non-linear regression was used to quantify certain aspects of the curve (α , $rETR_{max}$, I_k). The initial slope of the curve (α) provides an estimate of the light harvesting efficiency of photosynthesis. The asymptote of the curve, the maximum rate of photosynthesis ($rETR_{max}$), is a measure of the ability of the photosystems to utilize the absorbed light energy (Marshall et al., 2000). The minimum saturating irradiance (I_k) can be calculated by the following equation: $I_k = \alpha/rETR_{max}$, and is an indicator of the photoacclimation state of the plant (Ralph and Gademann, 2005). For the Diurnal Yield method, which is similar to the 'reconstructed' light curve method (Levy et al., 2004), effective quantum yields (Φ_{PSII} ; the first yield measurement of the RLC taken before actinic light is applied) and ambient irradiances were measured throughout the sample period (~ 0800 to 1700 h). Then $rETR$ s were calculated using each Φ_{PSII} measurement and the ambient irradiance at the time

of measurement. Non-linear regressions of the subsequent $rETR$ s and ambient irradiances were also calculated. All non-linear regressions were done in Sigmaplot 9.0 using a hyperbolic tangent function (Platt et al., 1980).

2.4. Statistical analysis

Light curves generated by the Diurnal RLC and Diurnal Yield methods were compared within each basin and between years (2002 and 2004). In order to compare the two methods and assess their ability to predict the relationship between calculated $rETR$ and ambient irradiance (PAR), non-linear (hyperbolic tangent) regressions were calculated. Linear regressions of the residuals of each non-linear regression and the independent variable (PAR) were also calculated to determine if either model exhibited irradiance-based bias. In order to determine if the two methods were significantly different from each other a linear regression of the difference between the data sets was plotted against the independent variable (PAR).

3. Results

Photosynthetic versus irradiance (P–E) curves constructed for the 10 basins sampled in FHAP during 2002 and 2004 enabled the comparison of the Diurnal RLC and Diurnal Yield methods. A linear regression of the difference between the calculated $rETR$'s of the two methods (Diurnal RLC–Diurnal

Yield) and the independent variable (PAR) determined the curves generated from the two methods were statistically different from one another ($r^2 = 0.785$, $P < 0.001$) and the difference depended on irradiance level in a strongly linear fashion (data not shown). Linear regressions of the residuals of each individual P–E curve plotted against the independent variable (ambient PAR) indicated no irradiance-based bias in the model fit for either method (data not shown). All non-linear regressions, which generated the P–E curves for the two methods, were significant (Table 1). Regressions of curves constructed with the Diurnal Yield method always had higher r^2 than the Diurnal RLC method (Table 1).

The P–E curves generated for the Diurnal RLC and Diurnal Yield methods exhibited consistently different patterns in all of the basins. For all basins and between both years, the two methods also suggested different relationships between electron transport rates (rETR) and high irradiance resulting in different calculated values of $rETR_{max}$ and I_k , but predicted generally similar values of α (Table 1). With the Diurnal Yield method, rETR increased as irradiance increased, which resulted in very high electron transport rate, $rETR_{max}$ (417–1715) and I_k (1045–4681 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) values, which were sometimes not calculable because of the linearity of the response (RAB in 2002 and RKN both years, Table 1, Fig. 1). In contrast, the P–E curves generated with the Diurnal RLC method

generally reached an asymptote at higher irradiances and resulted in much lower calculated values of $rETR_{max}$, which ranged from 100 to 200. The calculated initial slopes (α) from both methods were comparable within most basins, though the Diurnal Yield method usually had slightly higher α (Table 1). Calculated values of I_k were substantially higher (ranging from 1045 to 4681 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) with the Diurnal Yield method than the Diurnal RLC, which ranged from 0 to 1124 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Table 1). In one basin (CRN) the regression for the Diurnal RLC method in 2004 calculated an unrealistically high α (1651.31) and an I_k of 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Table 1). This reflected the reverse (upward) curvature of the rate of linear electron transport (ETR) versus PAR curve, which may be an artifact of the non-linear model fitting (data not shown). The mean parameters (α and $rETR_{max}$) and calculated I_k , for the RLC measured throughout each sample period, from which both diurnal methods were derived are also presented in Table 1. The calculated initial slopes (α) among curves produced with the measured (RLC) and both derived (Diurnal RLC and Diurnal Yield) methods were similar. However, only the Diurnal RLC method produced estimates of $rETR_{max}$ and I_k that were generally comparable to the mean of the daily RLCs (Table 1).

Examples of curves produced by the Diurnal Yield and Diurnal RLC methods for three of the ten basins sampled are

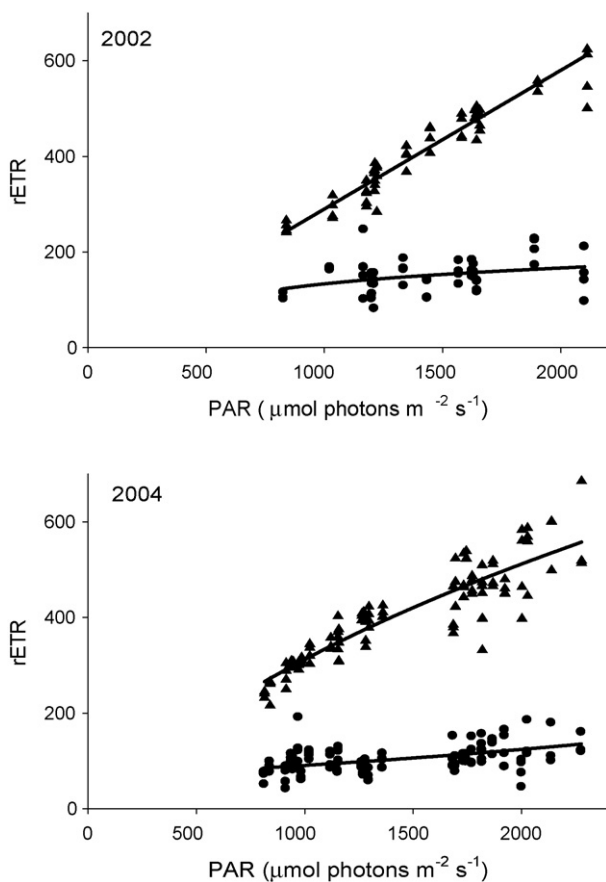


Fig. 1. *Thalassia testudinum*: Diurnal Yield (\blacktriangle) calculated rETR vs. irradiance (PAR $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and Diurnal RLC (\bullet) interpolated rETR vs. irradiance (PAR) for Rankin Lake (RKN) in 2002 and 2004.

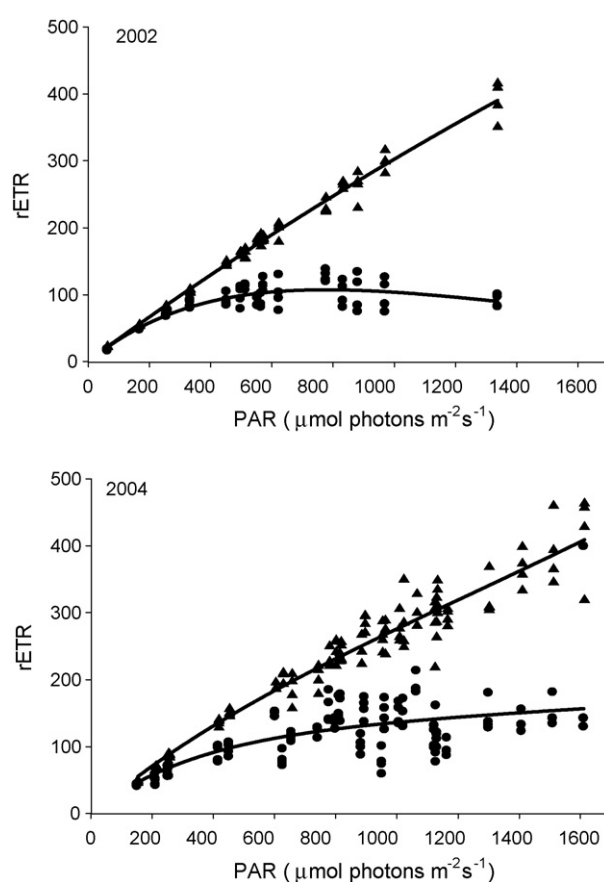


Fig. 2. *T. testudinum*: Diurnal Yield (\blacktriangle) calculated rETR vs. irradiance (PAR $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and Diurnal RLC (\bullet) interpolated rETR vs. irradiance (PAR) for blackwater sound (BLK) in 2002 and 2004.

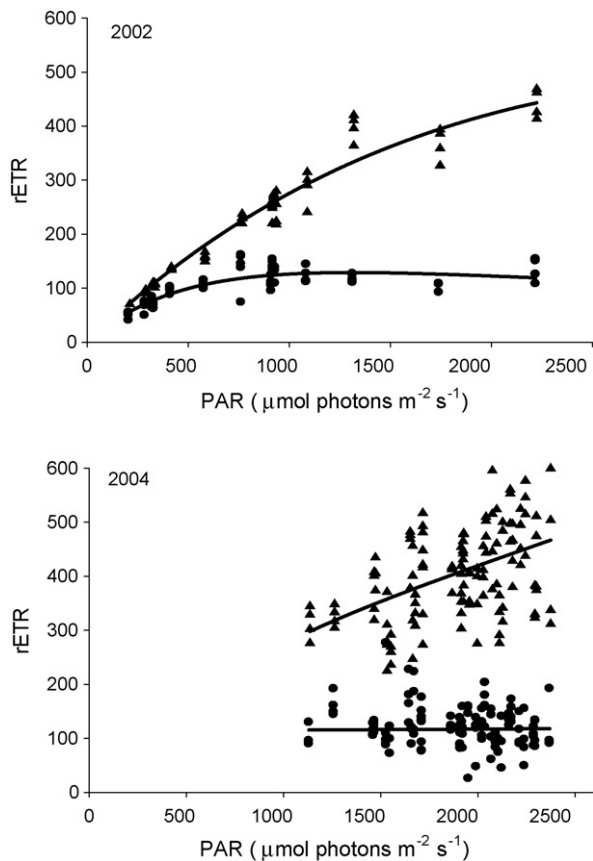


Fig. 3. *T. testudinum*: Diurnal Yield (\blacktriangle) calculated rETR vs. irradiance (PAR $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and Diurnal RLC (\bullet) interpolated rETR vs. irradiance (PAR) for eagle key basin (EAG) in 2002 and 2004.

shown in Figs. 1–3. These basins exhibited representative characteristics of the curve shapes produced by the two methods. In most of the basins the two methods suggested similar physiological condition of *T. testudinum* in both years (shown in Fig. 1 (RKN) and Fig. 2 (BLK)); also in MAD, RAB, CRN, TWN, and WHP), while in others there was substantial inter-annual variation (shown in Fig. 3 (EAG); also in JON and CAL). This was mainly due to differences in irradiances when fluorescence measurements were taken. The ambient irradiance measured at the seagrass canopy varied among basins and between years, but generally, irradiances during sampling were higher in 2004 (Fig. 4). The increase in ambient irradiance in 2004 had a greater effect on the P–E curves generated by the Diurnal RLC method, generally resulting in decreased α and increased I_k values from 2002 to 2004 (Table 1). In a few basins all fluorescence measurements were obtained at relatively high irradiances ($>1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, MAD and RKN in both years and EAG in 2004, Fig. 4). In these cases, the Diurnal RLC method generated P–E curves that were almost linear with near-zero slopes (shown in Fig. 1 for RKN and in Fig. 3 for EAG in 2004). Without measurements at lower irradiances this method was unable to generate realistic initial slopes. The resulting curves had unrealistically high (EAG in 2004) or relatively low (MAD and MAD) α values and very low (EAG in 2004) or high (RKN and MAD) I_k values; rETR_{max} estimates seemed unaffected by these conditions (Table 1).

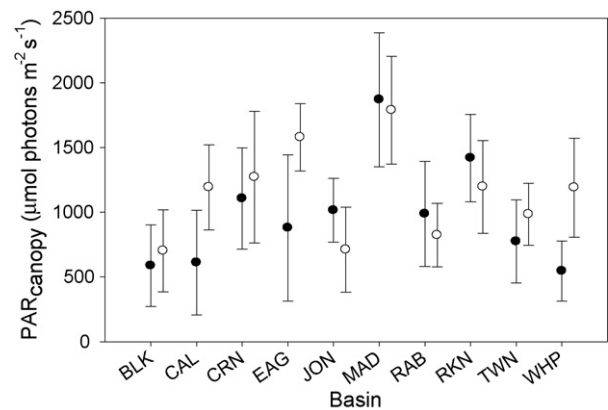


Fig. 4. Mean (\pm S.D.) ambient irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) at the seagrass canopy ($\text{PAR}_{\text{canopy}}$) measured in 10 basins sampled within Florida Bay in 2002 (\bullet) and 2004 (\circ).

4. Discussion

Our results indicate that the Diurnal Yield and Diurnal RLC methods suggest very different relationships between photosynthesis and irradiance for *T. testudinum*. Although there was a degree of inter-basin and inter-annual variation in the generated curves, some differences between the two methods were consistent. The Diurnal Yield method always predicted much higher photosynthetic capacities (rETR_{max}) and saturation irradiances (I_k) than the Diurnal RLC method. Generally, both methods provided comparable values for photosynthetic efficiency (α) though there was deviation from this trend in basins where benthic irradiances were high throughout the entire sample period. The two methods use the same RLC data taken throughout each sample period, yet they predict very different photosynthetic characteristics.

Both diurnal light curve methods are based on the assumption that effective quantum yield (Φ_{PSII}) measurements can be used to calculate the rate of linear electron transport (ETR). For certain seagrass species, calculated ETRs based on fluorescence measurements have a linear relationship with O_2 evolution, but for other species they only correlate at lower irradiances, with increasing discrepancies at higher irradiances (Beer et al., 1998; Beer and Björk, 2000). Without knowledge of the actual amount of light being absorbed, fluorescence measurements can only be used as an approximation for electron transport (i.e., rETR, Beer et al., 1998; Runcie and Durako, 2004; Saroussi and Beer, 2007). Although seagrass leaves exhibit relatively uniform light harvesting capabilities across varying depths, water quality and morphologies (Cummings and Zimmerman, 2003; Enríquez, 2005; Durako, 2007), the assumption that light absorption is constant for leaves growing in different microclimates across the landscape in Florida Bay may not be true. Therefore, the information provided by these two methods, as compared here using a constant absorptance factor of 1, represents an integrated approximation of landscape-level photosynthetic characteristics.

In P–E curves generated with the Diurnal Yield method, rETR continually increased with irradiance without reaching an asymptote, resulting in unrealistic predictions of rETR_{max} and I_k . This method uses the first quantum yield (Φ_{PSII})

measurement taken in each RLC to calculate rETR. Because the leaf blade is covered with the dark leaf clip for a few seconds before the first yield measurement is taken, this results in a quasi-darkness yield value (Ralph and Gademann, 2005), which allows some time for rapid re-oxidation of the primary electron acceptor (Q_A) and thus results in the highest quantum yield measurement taken during the RLC (Ralph and Gademann, 2005). Using ambient irradiances to calculate rETR, the Diurnal Yield method assumes that all of the photon energy captured by chlorophyll *a*, besides that re-emitted as fluorescence, is used for photochemistry. This may not be so because of the effects of non-photochemical quenching, which may be a major contributor of energy dissipation in high-light environments (White and Critchley, 1999; Marshall et al., 2000; Runcie and Durako, 2004; Ralph and Gademann, 2005). Also, the increase in irradiance in the bay from sunrise to noon was disproportionately larger than the decrease in effective quantum yield (Φ_{PSII}) measured during the mid-day (Belshe et al., 2007). Because these relatively high effective quantum yields are used as a multiplicand in the calculation of the rETR's, this results in disproportionately high values of rETR even though the efficiency of the system to utilize light decreases at high irradiances.

Oxygen-based values of photosynthetic capacity reported for *T. testudinum* in Florida Bay range from 171 to 256 $\mu\text{g O}_2 \text{g}^{-1} \text{dry wt min}^{-1}$ with saturation irradiances (I_k) ranging from 357 to 438 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fourqurean and Zieman, 1991). This O_2 -based capacity roughly translates to an ETR_{max} range of about 50–75 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, assuming it takes four electrons to produce one O_2 molecule in photosynthesis, a leaf absorptance of 0.47 (Cummings and Zimmerman, 2003) and specific leaf area of 155 $\text{cm}^2 \text{g}^{-1} \text{dry wt}$. (Enríquez et al., 2002). These O_2 -based ETR_{max} values are from slightly below, to about half, the range of values we report for both the Diurnal RLC method and the average of the RLCs for each day, assuming an AF of 1 (Table 1). The O_2 -based I_k values are generally very similar to the range of values calculated using both the Diurnal RLC method and the average of the RLCs for each day (Table 1). In contrast, calculations of rETR_{max} and I_k based on fluorescence measurements for the Diurnal Yield method were much higher, ranging from 417 to 1529 for the former and 1045–4681 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for the latter (Table 1). Because of the unrealistically high rETR_{max} and I_k values, we believe that the Diurnal Yield method does not provide a good indication of photosynthetic capacity for *T. testudinum* in Florida Bay. When comparing the Diel Yield curves to curves calculated from O_2 evolution, Longstaff et al. (2002) found that they only correlated at lower irradiances. The Diel Yield-based ETR curve continued to increase as irradiance rose while the O_2 -based curve tended toward asymptote. This indicated that an increased number of electrons were flowing through the photosystems for every O_2 evolved at higher irradiances (Longstaff et al., 2002). This could be due to increased photorespiration (Beer et al., 1998) or an increase in alternative forms of energy dissipation, such as non-photochemical quenching (Longstaff et al., 2002). At higher irradiances similar amounts of fluorescence may be

detected without increases in photosynthesis because of increased non-photochemical quenching (Levy et al., 2004; Schreiber, 2004).

With the Diurnal RLC method, the RLCs produced from the instrument-applied actinic irradiances were used to interpolate the rETR at the measured ambient irradiance taken at the time of the RLC. As actinic irradiances increase during a RLC, photochemical quenching decreases and non-photochemical quenching increases, due to an accumulation of electrons on the PSII acceptor side (White and Critchley, 1999; Schreiber, 2004; Ralph and Gademann, 2005). This results in a reduction of fluorescence and photochemical quenching as irradiance increases. Therefore, the interpolated rETR from this method is generated from actual measured responses of fluorescence to various light levels, which incorporates changes in photochemical and non-photochemical quenching. As a result, we believe that it is a more accurate representation of *T. testudinum*'s photosynthetic responses to irradiance.

In this study, fluorescence-based diurnal light curves generated from the Diurnal Yield and Diurnal RLC methods yielded different trends from those reported by Longstaff et al. (2002). They reported that the two methods provided comparable ETR_{max} estimates, but differing α , even though their curves resembled ours. A major difference between our experimental designs was the ambient irradiances in which the fluorescence measurements were taken. They reported a daily maximum irradiance of 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, while we measured irradiances that were up to six times as high (Fig. 4). Ambient irradiances were used in the calculations of rETR and P–E curves for both methods. Because of the high-light conditions ($>I_k$ all day) at basins like Madeira Bay and Eagle Key in 2004, which reflect the very shallow nature and clear water of some areas in Florida Bay, miscalculations of photosynthetic efficiency and, consequently, I_k resulted. In situations where irradiances were always high during our measurements this resulted in a relative absence of data points for the initial part of the curves. Even though the Diurnal RLC method predicted more realistic rETR_{max} and I_k , this method greatly overestimated, or underestimated, α in high irradiance conditions. With this method rETR is interpolated from RLCs that do reach saturation and level out. Therefore, the interpolated rETRs at high irradiances coincide with the saturated part of the curve and when irradiances are all $>I_k$, nearly flat (slope approaching 0) linear P–E curves result. It was largely because of the near linearity of the response of rETR and irradiance with the Diurnal Yield method that a reasonable slope (α) could be mathematically calculated from this model, for all basins and light conditions.

As determined by r^2 of the regressions, the Diurnal Yield method seemed to better predict the relationship between rETR and irradiance, although this method produced unrealistically high estimates of photosynthetic rates at high irradiances. In contrast, the Diurnal RLC method generally produced predictions of photosynthetic capacity similar to those predicted from O_2 methods; it was unable to predict photosynthetic efficiencies only when ambient irradiances were continuously high during the period of sampling ($>I_k$).

Sampling earlier or later in the day when irradiances are reduced should overcome this limitation, but may not be possible for some field-sampling programs for safety and navigational reasons. An alternative is to use the original data from the RLCs, which provide an actual measured photosynthetic response to lower light levels. Since the initial slopes of RLC's exhibit diurnal variation (White and Critchley, 1999; Belshe et al., 2007), calculating the mean of the slopes obtained throughout the day may give a reasonable approximation of the photosynthetic response of the seagrass to the diurnal range of light levels for a particular basin. We believe that with these further calculations, RLC methods can be used to approximate the relationship between irradiance and electron transport. Based on the recent RLC results of Saroussi and Beer (2007), we further recommend, if possible, measuring absorption factor (AF, Beer and Axelsson, 2003) or leaf absorptance (Cummings and Zimmerman, 2003; Runcie and Durako, 2004; Enríquez, 2005; Durako, 2007) to more accurately estimate electron transport rates.

Acknowledgements

This project was supported by the United States Geological Service Biological Resources Division, Everglades National Park and the National Undersea Research Center. Additional support was provided by the University of North Carolina Wilmington, Department of Biology and Marine Biology and the Center for Marine Science. The authors thank Jennifer Kunzelman and Brooke Landry from UNCW and Manuel Merello, Donna Berns, Kerri Fareq and Dr. M.O. Hall from the Florida Marine Research Institute for their help and support in the field.

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